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MINIREVIEW

Mechanisms by Which Ambient Humidity May Affect Viruses in Aerosols

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Many airborne viruses have been shown to be sensitive to ambient humidity, yet the mechanisms responsible for this phenomenon remain elusive. We review multiple hypotheses, including water activity, surface inactivation, and salt toxicity, that may account for the association between humidity and viability of viruses in aerosols. We assess the evidence and limitations for each hypothesis based on findings from virology, aerosol science, chemistry, and physics. In addition, we hypothesize that changes in pH within the aerosol that are induced by evaporation may trigger conformational changes of the surface glycoproteins of enveloped viruses and subsequently compromise their infectivity. This hypothesis may explain the differing responses of enveloped viruses to humidity. The precise mechanisms underlying the relationship remain largely unverified, and attaining a complete understanding of them will require an interdisciplinary approach.

Inquiry into the influence of ambient humidity on viral disease transmission began decades ago, and the (re)emergence of pandemic influenza, H5N1 influenza, and severe acute respiratory syndrome (SARS) has rekindled interest in this topic (4, 28). Many airborne viruses have been shown to be sensitive to ambient humidity (41, 50). A thorough understanding of this phenomenon may provide insight into the temporal and spatial distribution of diseases. For instance, studies have repeatedly suggested ambient humidity as an important environmental determinant in the transmission of influenza in temperate regions (21, 47, 48). Knowing how to optimize humidity so as to minimize virus survival may have practical implications for disease prevention (35).

The relationship between humidity and the survival of airborne viruses appears to be straightforward, but contradictions in the literature on the relationship (Table 1) remain unexplained. Moreover, despite decades of investigation, the question of why a virus encased in an aerosol would be affected by ambient humidity remains largely unanswered. The answer to this question is essential to the understanding of the interplay between humidity and viruses. It may help determine the effect of humidity under natural conditions and identify factors that may confound experimental studies. However, mechanisms that have been proposed in the literature are usually mentioned only in passing and largely remain unproven.

In this study, we review multiple hypotheses, including water activity, surface inactivation, and salt toxicity, to explain the relationship between humidity and virus viability in aerosols (11). We assemble principles and new findings from multiple disciplines, including virology, aerosol science, chemistry, and physics, to assess the evidence and limitations for each mechanism. Further, we propose a new mechanism to account for the differing responses of enveloped viruses to humidity.

GENERAL RELATIONSHIPS BETWEEN RELATIVE HUMIDITY AND VIABILITY

The relationship between relative humidity (RH) and viability has been thoroughly reviewed in a WHO report by Sobsey and Meschke (50). In general, enveloped viruses, which contain a lipid membrane, survive better at lower RH, while nonenveloped ones tend to be more stable at higher RH (50). However, there are many exceptions that remain unexplained (Table 1). Rous sarcoma virus (RSV) and infectious bovine rhinotracheitis virus (IBRV), both enveloped, were observed to be more stable at higher RH, and pigeon pox virus, also enveloped, was reported to be insensitive to RH (51, 60). There are also exceptions among nonenveloped viruses, for instance, feline calicivirus and vesicular exanthema virus (12).

RH, EVAPORATION, AND WATER ACTIVITY

RH is defined as the ratio of the actual water vapor pressure to the saturation vapor pressure of ambient air. When an aerosol transitions from higher to lower RH (e.g., when it is released from the respiratory tract into ambient air), it is subject to evaporation due to the vapor pressure gradient between its surface and ambient air. As evaporation proceeds, the water vapor pressure at the surface, which is proportional to the molar fraction of water in the aerosol (Raoult's Law), decreases because water is lost to evaporation while solutes such as salts and proteins remain. Evaporation ceases when the vapor pressure at the aerosol's surface is reduced to that of ambient air, a point at which the water activity of the aerosol equals the ambient RH (42). The extent to which an aerosol evaporates depends on its solute content and RH, and the final size can be calculated using mathematical models based on thermodynamics and fluid mechanics (32, 39, 44). Small droplets (less than ~30 μm) reach their equilibrium size in less than 1 s (22, 36).

It has been hypothesized that removal of structural water molecules from the virus's capsid can lead to inactivation (12). The hypothesis has been tested with bacteria (59); however, whether it applies to viruses, which have completely different structures, has not been shown.

There is evidence suggesting that abrupt rehydration of non-

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TABLE 1 Relationship between RH and survival of various viruses in aerosols[a]

Virus category and name	Fusion pH [reference(s)]	Survival at each RH[b]			Notes	Spraying medium	Reference(s)
		Low	Middle	High			
Enveloped viruses							
Category I							
Langat	Low pH (<6) (52)	++	−	+	Viability highest at ~20% RH, lowest at 40–60% RH, and medium at RH of >70%; polyhydroxy compounds (including 5% inositol, sorbitol, and glucose) protected the virus at 50% RH at 5 min to 3 h	Culture medium (salts and proteins)	1
		++	−	++	Viability highest at ~20% RH, lowest at 50% RH, and medium at RH of >70% at 1 h	Salt solutions (NaCl, KCl, LiCl)	1
		++	++	+	Viability decreased with increasing RH at 1 and 3 h, especially at 3 h	Desalted medium (with proteins)	1
		++	++	++	High viability across ~20–95% RH at 1 h	Desalted and deproteinized suspension	1
		++	++	−	Viability higher at ~20–70% RH and lower at RH of >~70% at 1 h	Desalted and deproteinized suspension plus 0.1% BSA	1
Semliki Forest virus	Low pH (61)	++	++	−	Viability higher at ~20–60% RH and lower at ~85% RH at 24 h	Desalted suspension	1
		++	−	−	Viability higher at 20–40% RH and lower at 50–85% RH at 24 h	Desalted suspension plus 5% NaCl	1
Influenza (PR8)	Low pH (<5) (25)	+	−	−	Viability higher at 15–40% RH and 10 times lower at 50–95% RH	1 part allantoic fluid plus 1 part 2% peptone	20, 21
Influenza A (PR8)	Low pH (<5) (25)	+	−	−	Viability decreased with increasing RH	Allantoic fluid diluted 1:8 or 1:10 in casein McIlvaine's buffer (pH 7.2)	18
Influenza A (W.S. strain)	Low pH (<5) (25)	+	−	+	Viability highest at 30–34% RH, lowest at 58–60% RH, and medium at 66–70% RH	Allantoic fluid in 0.1 M Sorensen's phosphate buffer (pH 7.1)	49
Influenza A (WSN strain)	Low pH (<5) (25)	+	−	+	Viability highest at RH of <40%, lowest at 40–60%, and medium at RH of >60%	MEM; MEM plus 0.1% BSA; allantoic fluid	45
SARS CoV	Low pH (58)	++	−	−	Inactivation on surfaces, done with droplets; stable at 40–50% RH and more rapidly inactivated at higher RH	Cell culture maintenance medium	4
Venezuelan equine encephalomyelitis	Low pH (6)	?	?	?	Initially, viability lower at 20% RH and higher at 50% and 80% RH, but at 23 h, viability higher at 20% RH	Chicken embryo prepn	18, 19
Category II							
Rous sarcoma virus	Neutral pH (17)	+	−	++	Viability decreased from 10–30% RH, was minimal at 30% RH, and then increased with increasing RH at RH of >30%; highest at ~100% RH	Water	60
		++	++	++	Viability high at all RHs	6% i-inositol	60
		++	−	−	Viability decreased with increasing RH	0.005 M citrate buffer	60
Bovine rhinotracheitis virus	Neutral pH (9)	−	−	+	Viability lower at 10% and 35% than at 90% RH	Culture medium (with both salts and proteins)	51
Category III							
Vaccinia virus	Both neutral and low pH (2, 26)	+	+	+	Viability similar at 20–84% RH and slightly lower at ~80% RH	McIlvaine's citric acid/disodium phosphate buffer plus 1% dialyzed horse serum	18, 19
Pigeon pox virus	Either, depending on virus strain and cell line (34)	++	++	++	Viability high at all RHs	Water	60
Nonenveloped viruses							
Polio (type I)	NA	+	−	++	Viability medium at 18–36% RH, lowest at 49–51% RH, and highest at 64–81% RH	Earle's saline	18, 19
		−	−	++	0% viability at 20% and 50% RH, 50% viability at 80% RH	Water	19
		−	−	++	0% viability at 20% and 50% RH, 89% viability at 80% RH	0.5% gelatin	19
		−	−	++	0% viability at 20% and 50% RH, 23% viability at 80% RH	Phosphate buffer	19
		−	−	++	0% viability at 20% and 50% RH, 32% viability at 80% RH	0.1% cysteine	19
		+	ND	+	1% viability at 20% RH, 0.2% viability at 80% RH	0.5% NaCl	19
		−	−	+	0% viability at 20% and 50% RH, 4% viability at 80% RH	0.63% Na_2SO_4	19

		+	+	+	4% viability at 20% RH, 2% viability at 50% RH, and 9% viability at 80% RH	0.65% KCl	19
Polio (types I and III)	NA	−	−	+	Viability higher at higher RH	1 part culture medium (Hanks salt solution plus lactalbumin-hydrolysate plus 5% horse serum) and 1 part 2% peptone	20, 21
Polio (type I)	NA	+	−	++	Viability medium at RH of <40%, lowest at 45–60% RH, and highest at RH of >60%	Clarified culture fluid	1
		+	−	++	Viability medium at RH of <60%, lowest at ~70% RH, and highest at RH of >80%	Deproteinized and desalted	1
		++	ND	ND	At 20% RH, when prehumidified before collection, viability 10 times higher than without prehumidification (29% ± 5% vs 3.2% ± 1.8%)	Clarified culture fluid	1
		ND	ND	++	At 70% RH, when added 5% NaCl, the virus survived better than in desalted and deproteinized suspension	Deproteinized and desalted suspension plus 5% NaCl	1
Feline calicivirus	NA	+	−	−	Viability higher at RH of <30% and lower at 40–80% RH	Eagle's medium	12
Vesicular exanthema virus	NA	+	−	+	Viability higher at 20% RH, decreased with increasing RH, lowest at 60%, and then increased at RH of >60%	Eagle's medium	12
Coliphages							
T7	NA	−	−	++	Viability decreased with decreasing RH	Clarified lysates (with salts and proteins)	1
		+	−	++	Viability medium at 20–50% RH, lowest at 55–75% RH, highest at RH of >75%	Desalted medium; desalted and deproteinized medium	1
		++	ND	ND	At 20% RH, when prehumidified before collection, viability 1,000-fold higher than without prehumidification (28% ± 19% vs 0.02% ± 0.01%)	Clarified lysates	1
		ND	ND	++	At 70% RH, when added 5% NaCl, the virus survived better than in desalted and deproteinized suspension	Deproteinized and desalted suspension plus 5% NaCl	1
T5	NA	+	−	−	Viability decreased with increasing RH; lower in saline than in other media	1% peptone, allantoic fluid, distilled water; physiological saline	19

[a] CoV, coronavirus; ?, results inconclusive; NA, not applicable; ND, not determined; MEM, minimum essential medium.

[b] This column indicates various viruses' relative preferences for a relative humidity (RH) range. RH levels: low, below ~50%; middle, ~50% to ~70%; high, above ~70%. These cutoffs are only approximate, and the true values vary widely depending on experimental conditions and the specific virus of interest. "++" denotes high viability, indicating that generally over 10% viability was recovered after exposure to that RH; "+" denotes relatively high viability, but viability was about one order of magnitude lower than "++"; "−" denotes low viability, generally 1 to 3 orders of magnitude lower than in the other two cases.

enveloped viruses affects their viability. Such a condition occurs, for example, during sample collection with an impinger when an aerosolized virus is impacted into the liquid. Benbough exposed aerosolized viruses to 20% RH and then subjected some samples to prehumidification at 100% RH prior to collection with an impinger (1). With prehumidification, recovery rates (± standard deviations) were much higher for nonenveloped viruses (T7 coliphage, 28% ± 19% with prehumidification versus 0.02% ± 0.01% without prehumidification; poliovirus, 29% ± 5% versus 3.2% ± 1.8%) but were unchanged for enveloped viruses (Semliki Forest virus [SFV], 32% ± 7% versus 42% ± 8%; Langat virus, 42% ± 10% versus 51% ± 12%) (1). In this experiment, it appears that the lipid membrane of enveloped viruses may have protected their capsids from damage due to changing humidity. Benbough attributed the inactivation of nonenveloped viruses to structural rearrangement during abrupt rehydration in the impinger collection medium and suggested that slower rehydration rates, such as those experienced during the prehumidification process, were more favorable for polioviruses and coliphages (1). Since RH is close to 100% within the respiratory tract (64), respiratory viruses are likely to undergo slow rehydration when being inhaled. Therefore, a more accurate simulation of the effect of RH on nonenveloped respiratory viruses, such as rhinovirus, may need to account for this effect.

Another study (8) suggested that the loss in viability of some coliphages during rehydration may be due to damage to coliphages' head-tail complex. Since the head-tail complex is unique to coliphages, the inactivation mechanisms for polioviruses and possibly other nonenveloped viruses might be different. Also, results suggest that some mechanisms may apply only to certain viruses due to their unique structure (e.g., the head-tail complex of coliphages). These findings caution against the use of tailed coliphages as a surrogate to study other viruses and/or extrapolating results based on them to other viruses.

SURFACE INACTIVATION

Viruses that partition on the surface of aerosols may be subject to damage due to surface tension, shear stress, and conformational rearrangement driven by hydrophobicity. Donaldson and Ferris (12) tested the viability of eight viruses in medium subject to aeration, which increased the viruses' exposure to the air-liquid interface. Results showed that enveloped viruses lost infectivity dramatically (up to four orders of magnitude) due to aeration, while the nonenveloped ones did not. The addition of 0.1% peptone reduced losses of enveloped viruses by less than one order of magnitude compared to the controls. The authors hypothesized that enveloped viruses were likely to accumulate at the surface of droplets, where "unbalanced forces" acting on the virions may be strong enough to produce inactivation through irreversible unfolding and rearrangement of molecules (12, 55).

RH can modulate the area of the air-liquid interface available for virus accumulation. The surface area (A) of an aerosol, if assumed to be spherical, scales with its diameter (d) squared ($A = \pi d^2$). Higher RH allows for larger final aerosol size and larger surface area due to less evaporation and, thus, greater potential for surface inactivation of hydrophobic lipid-containing viruses (i.e., enveloped viruses). Nonenveloped viruses are less likely to be affected. The addition of proteins to spraying medium may reduce the tendency of viruses to accumulate on the surface and, thus, diminish the loss of viability due to surface inactivation.

Studies have shown that unfolding of peptides and subsequent denaturing of proteins can occur at the air-water interface (16, 43). These findings suggest that surface inactivation may compromise the infectivity of enveloped viruses with surface glycoproteins. However, whether similar conformational changes indeed happen and, if so, whether they affect viability remain to be tested. In addition, this mechanism would only affect viruses at the aerosol's surface. Therefore, a more complete understanding of this mechanism will require quantitative investigation into the partitioning of viruses between the surface and the bulk of the aerosol. Furthermore, the fact that viability varies with RH despite the addition of hydrophobicity modifiers such as proteins for some enveloped viruses (1, 18) indicates that mechanisms other than surface inactivation are also influential.

EFFECTS OF SALTS

Salts and RH. Salts are common components in both physiological fluids and experimental media. In aerosols subject to evaporation, salts become more concentrated as water is lost. However, interactions among salts, water, and RH are dynamic, and concentrations of salts do not correlate with RH in a linear manner (5). A droplet of salt solution can lose all its water and crystallize at a low/medium RH unique to the salts it contains (i.e., efflorescence relative humidity [ERH]). On the other hand, many inorganic salts can spontaneously absorb water from the ambient air at a particular higher RH (i.e., deliquescence relative humidity [DRH]). Among the most prevalent salts in physiological solutions, NaCl has an ERH of 43% ± 3% and a DRH of ~75% and KCl has an ERH of ~59% and a DRH of ~84% (29, 46).

Effects of salts on enveloped viruses. In aerosols composed of mainly salt solutions, the viability of some enveloped viruses (e.g., SFV and influenza A virus [IAV]) has been shown to decrease with decreasing RH, reach a minimum at medium RH, and remain relatively high at RH of <~50% (1, 45, 49). Our prior work identified three regimes of viability and RH for IAV (W. Yang, S. Elankumaran, and L. C. Marr, submitted for publication). (i) The first relates to physiological conditions at RH close to 100%, where viability is well preserved. At RH close to 100%, evaporation is minimal, and concentrations of salts in the aerosol thus stay at levels close to physiological conditions, which are harmless to the viruses. (ii) The second involves concentrated conditions (~50% to near-100% RH), where viability decreases with decreasing RH in medium containing salts only. In this regime, evaporation is intense, and salts are concentrated enough to be toxic. The solution can even become supersaturated. The toxic effect of salts is supported by the finding that NaCl at concentrations in excess of 1 M (i.e., five times physiological levels and easily achieved in an aerosol following evaporation [39]) leads to significant changes to membrane structure and elasticity (38). (iii) The third involves dry conditions (<~50% RH), where salts crystallize and viability is maintained. When the RH is less than the ERH, salts crystallize, and thus, their toxic effects are eliminated. The trend in IAV viability that we observed in salt solutions (e.g., phosphate-buffered saline [PBS]) was in accordance with that reported by Shechmeister (49) and Schaffer et al. (45). Furthermore, we showed that addition of proteins to the medium altered the relationship such that it was similar to those reported by Hemmes et al. (21) and Harper (18). These findings thus resolve the conflicting conclusions from the four aforementioned studies (53, 63).

The precise mechanisms for the toxic effects of salts on envel-

oped viruses remain unclear. Past studies hypothesized that the toxic effect of chlorides was caused by Cl^- ions displacing bound water in membrane systems and consequently breaking down the lipoprotein of the virus envelope (1). However, a recent study (57) indicated that both Na^+ and Cl^- ions can interact with lipid bilayers. In addition, studies have shown that various cations (including Na^+, Ca^{2+}, and Mg^{2+}) can induce structural and mechanical changes in lipid bilayers through strong binding (7, 27). More experiments are warranted to pinpoint the interactions between salt ions and viruses. Nevertheless, current findings suggest that viral inactivation may be driven by functional alteration of the lipid membrane.

Effects of salts on nonenveloped viruses. While salts appear to be toxic to enveloped viruses, addition of salts to the medium has been shown to improve rather than reduce the viability of nonenveloped viruses, such as poliovirus, and T7 coliphage (1, 19), as shown in Table 1. To account for this interesting effect, Benbough (1) suggested that salts can slow the rehydration process during sample collection and thus reduce the chance of structural rearrangements that can be damaging in the capsid of nonenveloped viruses. Our interpretation of the enhanced viability is that salts can keep the aerosol and the virus in it from drying out completely at RHs above the ERH and thus reduce the chance of unwanted structural rearrangements during rehydration. This effect would benefit the nonenveloped viruses that need to retain their structural water; meanwhile, they are not susceptible to damage by salts, as are enveloped viruses.

Altogether, these results suggest that RH can define the virus's microenvironment in the aerosol, in which water and salt ions interact dynamically with the virus. However, some enveloped viruses have been shown to be stable at medium and high RHs, even though their lipid bilayers are similar to those of other enveloped viruses (51, 60). In addition, Benbough (1) showed that the viability of SFV remained lower at RHs of >60% than at RHs of <60% when salts were removed from the spraying fluid. These exceptions indicate that the toxic effect of salts alone may not account for the complete influence of RH.

CHANGES IN pH AND EFFECTS ON ENVELOPED VIRUSES

Hypothesis. To account for the aforementioned exceptions among enveloped viruses, we propose a new mechanism to explain the influence of RH on viability. Due to evaporation, the concentration of free H^+ ions in an aerosol would increase and, in turn, reduce the pH. As a result, the glycoproteins embedded within the membrane of enveloped viruses which are essential to viral attachment and entry to host cells can undergo structural rearrangements depending on their specific response to low pH. Accordingly, we hypothesize that RH can affect the viability of enveloped viruses by altering the pH in the aerosol, which, in turn, induces conformational changes to the viral glycoproteins and damage to viral infectivity.

RH and changes in pH. The magnitude of change in pH in an aerosol can be estimated through model calculations. Using a model based on Köhler theory (32, 63), we calculated the final size of a 10-μm droplet composed of 2.2 g/liter of KCl and 2.2 g/liter of proteins represented as bovine serum albumin (BSA) (similar salt and protein content to saliva [10, 31]). Results indicate that such a droplet can shrink to 0.24 of its initial diameter at 90% RH, corresponding to a 70-fold (i.e., $1/0.24^3$) increase in the concentration of free H^+ and a decrease in pH of 1.8 units, and to 0.17 of its initial diameter, with a decrease in pH of 2.3 units, at 60% RH. Additionally, H^+ tends to accumulate on the surface of droplets with a partition coefficient of 1.5 (i.e., ratio of surface concentration to bulk concentration) (40). This effect would further reduce the pH on the surface, where the enveloped viruses tend to accumulate (12), by 0.2 units compared to that within the droplet.

Enveloped virus fusion and pH. To initiate the replication process, enveloped viruses have to enter host cells through fusion. It generally follows one of two pathways, either direct fusion with the plasma membrane or fusion following endocytosis and intracellular trafficking, and some viruses are able to employ either pathway. Many enveloped viruses, such as RSV (17) and alphaherpesvirus (9), fuse directly with the plasma membrane at neutral pH. In contrast, others, such as IAV (25), Langat virus (a flavivirus) (52), and SFV (an alphavirus) (61), enter host cells through the endocytosis pathway; these viruses usually require low pH (5 to 6 or lower) to trigger the fusion process. For instance, IAV attaches to sialic acid-containing receptors via the hemagglutinin (HA) glycoprotein and is then internalized through endocytosis. Within the endosome, the HA glycoprotein undergoes an acid-catalyzed conformational rearrangement at a pH of <5, exposing the fusion peptide, and subsequently fuses with the endosomal membrane (3, 14). Low pH induces similar acid-catalyzed conformational changes in the viral glycoproteins for flavivirus and alphavirus (14, 24). By the same mechanism, acidification outside the host cell without the presence of the target membrane may induce conformational changes in the glycoproteins that, if irreversible, would inactivate the virus's fusion activity and, hence, infectivity (17, 30, 56). In contrast, pretreatment at low pH of a virus that does not require acid-catalyzed conformational changes preceding fusion generally does not compromise its infectivity (17, 30).

RH and conformational changes to surface proteins. Combining the analyses above, low RHs may induce evaporation, declines in pH, and in turn, conformational changes to surface proteins. At RHs above the ERH (so that viruses are still in solution), lower RHs would thus lead to larger decreases in pH, for example, 2.3 units at 60% RH or 1.8 units at 90% RH, according to our calculations. Since conformational changes to the viral glycoproteins are triggered below a threshold pH (e.g., pH 5 for IAV [25]), a slight decrease in pH near the threshold that corresponds to a specific RH may tip the balance and trigger the denaturing of glycoproteins.

A recent study seems to support our hypothesis. Imai et al. (23) found that an H5N1 IAV mutant with an additional mutation conferring a lower fusion pH threshold was able to replicate more efficiently in ferret nasal turbinates. The authors attributed this effect to the mutant's improved stability of the HA protein in an acidic environment (e.g., the pH in human nasal mucosas in which human IAVs primarily replicate is ~5.5 to 6.5 [13, 23]). This study indicates that pH may play an important role in the stability of viruses. Since the aerosols are generated from the virus's replication site, their pH is likely to be acidic and would become more so as the RH decreases. Thus, the viral membrane fusion proteins' sensitivity to pH may be a mechanism by which RH affects virus viability.

Our estimate of the shrinkage of a droplet and its change in pH is a simplification. The real change in pH following evaporation at a certain RH may be far more complicated due to interactions between different solutes, influence of the chemical composition

of the surrounding atmosphere, buffering effects of proteins, and heterogeneity in the spatial distribution of different solutes in an aerosol. In particular, proteins have repeatedly been shown to be protective for various enveloped viruses (1, 51, 54). Their buffering effects may explain, in part, this observation. Nevertheless, this analysis indicates that the change in pH associated with droplet evaporation at a specific RH may be a potential mediator of virus viability.

Findings on RH and viability fit with our hypothesis. We thus examine the relationship between RH and viability for various enveloped viruses and how each of them enters the host cell. Of the viruses studied in the literature (Table 1), we found that whether fusion requires low pH differentiates the responses of enveloped viruses to different RHs and appears to divide them into three categories (1). Viruses that require acidification before fusion are less stable at 50 to 90% RH than at RHs outside this range. Such examples include IAV (18, 21, 25, 45, 49), SFV (1, 61), Langat virus (1, 52), Venezuelan equine encephalomyelitis virus (6, 18, 19), and SARS coronavirus (4, 58). At ~50 to 90% RH, the aerosol generally does not dry out completely, yet evaporation is intense enough to lower the pH significantly. The low pH may compromise the viability of these viruses immersed within the aerosol. The lower the RH within this range, the lower the pH of the aerosol and the more likely the viruses are damaged (2). Viruses that fuse at neutral pH are more stable at 50 to 90% RH. Such examples include RSV (17, 60) and IBRV (9, 51). Changes in pH are similar to those in the first category; however, since viruses in this category are insensitive to low pH, they are less likely to be affected. The lower viability of these viruses at low RH (<50%) may be due to other mechanisms unique to these viruses (3). Viruses that can enter the host cell by both pathways, either at low or neutral pH, appear to be insensitive to RH. Viruses that fall into this category include vaccinia virus (2, 18, 26) and pigeon pox virus (34, 60). These results seem to support our hypothesis.

Which pathway a virus takes to enter a host cell and whether fusion requires low pH may be specific to the cell line. The herpes simplex virus is an exemplar. Early studies using HEp-2 and Vero cells indicated that penetration of cells by herpes simplex virus can be initiated by receptor binding and pH-neutral fusion with the cell surface (15, 62); however, more-recent studies reported another major pathway by endocytosis that requires low pH using HeLa and Chinese hamster ovary cells (33, 37). This sensitivity may confound the relationship with RH for viruses whose entry pathway depends on the host cell. On the flipside, experiments using viruses with multiple entry pathways and their corresponding cell lines may provide an avenue to test our hypothesis.

CONCLUDING REMARKS

The relationship between aerosolized viruses and RH is probably a combined function of properties of the virus and the interactions among the virus, solutes, and water molecules. RH may affect the viability of a virus in aerosols by controlling the amount of water retained, the equilibrium size of an aerosol and, thus, its surface area, the concentrations of solutes, and its pH.

This study focuses on the mechanisms underlying the effect of RH on the survival of airborne viruses. However, it is worth noting that temperature is another factor that may influence the relationship beyond the direct effect of temperature on virus viability. RH is a function of temperature because ambient temperature determines the saturation vapor pressure of water. Consequently, the relationship between virus survival and RH should be evaluated at a constant temperature in order to avoid potentially confounding effects.

There are still large gaps in the literature. A complete understanding of underlying mechanisms will require more in-depth studies with collaboration across disciplines. A better understanding of the interplay between environmental factors and viruses will hopefully lead to improved prevention and control of viral infectious diseases.

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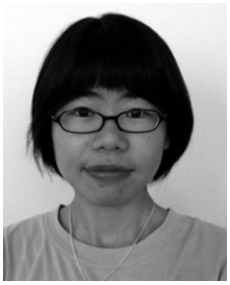

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